STEM CELLS

Reprogramming with low pH

temporary exposure to low pH can quickly reprogramme cells to pluripotency

Reprogramming of somatic cells to pluripotency is generally achieved by perturbing nuclear functions, for example, by nuclear transfer or by introducing exogenous transcription factors. Now, Obokata *et al.* show that lineage-committed lymphocytes and other cells can be converted to a pluripotent state by altering only the properties of their extracellular environment. They termed this unusual fate-conversion phenomenon ‘stimulus-triggered acquisition of pluripotency’ (STAP).

The authors used mouse CD45⁺ leukocytes, isolated from postnatal (1-week-old) spleens, carrying *GFP* under the control of the octamer-binding protein 4 (*Oct4*) promoter. These cells normally do not express pluripotency-associated markers and, consistently, did not express *Oct4* when cultured in a medium containing leukaemia inhibitory factor (LIF) and B27, which is used for the maintenance of pluripotent mouse cells. However, a 30 minute incubation in a low-pH medium (pH 5.4–5.8) led to the appearance of several clusters of cells expressing *Oct4* (*Oct4*-GFP⁺ cells). After culture for a further 7 days in medium with LIF and B27, most of the cells died. However, approximately one-third of the surviving cells expressed the *Oct4* pluripotency marker and lost expression of the CD45 marker (*Oct4*-GFP⁺ CD45⁻ cells), which is indicative of dedifferentiation. Importantly, no cell divisions were observed after the low-pH treatment, which suggests that *Oct4*-GFP⁺ cells were generated *de novo*, rather than through selection followed by amplification of stress-resistant cells.

The *Oct4*-GFP⁺ cells expressed several pluripotency-related marker genes (including *Nanog*) and marker proteins, similarly to embryonic stem (ES) cells. Moreover, the promoter regions of *Oct4* and *Nanog* were extensively demethylated in low-pH-induced *Oct4*-GFP⁺ cells, which indicates that key pluripotency genes underwent substantial epigenetic reprogramming.

When carrying out assays to test the differentiation capacity of *Oct4*-GFP⁺ cells, the authors found that cells that expressed GFP at high levels could give rise to three germ-layer derivatives *in vitro* and, when injected into mice, they formed teratomas. Thus, committed somatic cells can be converted into a state of pluripotency by external stresses, such as low pH; the resulting cells were referred to as ‘STAP cells’.

Blastocyst injection of STAP cells led to chimaeric embryos with STAP cells contributing to all the tissues examined. Furthermore, the offspring of chimaeric mice included animals derived from STAP cells, demonstrating their germline transmission, which is a strict criterion for pluripotency. However, when compared to mouse ES cells, the authors observed that STAP cells differed in that they had limited capacity for self-renewal in LIF-containing medium. This limitation was circumvented by addition of adrenocorticotrophic hormone (ACTH) to the medium, which converted STAP cells into proliferative cells. Such *in vitro* expandable cells were named ‘STAP stem cells’.

In a separate study, Obokata *et al.* further examined the developmental potential of STAP cells. This was prompted by the observation that,

in blastocyst injection assays, STAP cells contributed to both the embryonic and placental membranes. This was unexpected, as injected ES cells are usually found to only contribute to the embryonic portion of chimaeras, not the placental portion. Intriguingly, STAP stem cells lost the capacity to differentiate into extra-embryonic tissues. However, when primary STAP cells were cultured in a medium containing fibroblast growth factor 4 (FGF4), they could be converted into proliferative cells capable of contributing to placental tissues. It is important to note that such ‘FGF4-induced stem cells’ differ from trophoblast stem cells (TSCs), the precursors of differentiated placental cells, by also contributing to embryonic tissues and by continuing to express *Oct4*.

Together, these studies show that temporary exposure to low pH can quickly reprogramme cells to pluripotency. Converted cells can differentiate into all somatic cell, germ cell and extra-embryonic lineages, depending on the culture medium used for expansion. Importantly, as they are different from ES cells and TSCs and have a wider differentiation potential, these cells constitute a distinct state of pluripotency. The authors suggest that transient exposure to strong stimuli might ‘unlock’ somatic cells from lineage commitment, revealing a surprising degree of plasticity. The exact mechanisms underlying this unlocking remain to be elucidated, and intriguing questions arise such as for what purpose somatic cells may latently possess a self-driven ability for reprogramming.

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ORIGINAL RESEARCH PAPERS Obokata, H. *et al.* Stimulus-triggered fate conversion of somatic cells into pluripotency. *Nature* **505**, 641–647 (2014) | Obokata, H. *et al.* Bidirectional developmental potential in reprogrammed cells with acquired pluripotency. *Nature* **505**, 676–680 (2014)

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